

The Role of Sertoli Cells Adjacent to Efferent Ductules in Sperm Transport in *Rana porosa porosa*

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ABSTRACT—Ultrastructural changes in Sertoli cells adjacent to efferent ductules were observed before and during sperm transport in *Rana porosa porosa*. In control frogs, the lumina of seminiferous tubules were not linked with the lumina of efferent ductules. Sertoli cells adjacent to efferent ductules were in close contact with the ductule cells, and no tubule lumen opened into the efferent ductules. After hCG treatment, dilation of the Sertoli cells was observed, and the lumina of seminiferous tubules and the lumina of efferent ductules were linked. Spermatozoa were then seen in the efferent ductules. Thus, the present observations indicate that Sertoli cells adjacent to efferent ductules act as a valve-like device for sperm transport from the seminiferous tubules to the efferent ductules in frogs.

INTRODUCTION

The significance of testicular contractile peritubular myoid cells in sperm transport has been pointed out in mammals. The intratesticular excurrent duct system is composed of the rete testis, tubuli recti and the terminal segment of the seminiferous tubules [1], and the last one acts as a valve-like device for the transport of sperm and fluid [2]. In anurans, however, little information has been available on the mechanism of sperm transport. Only a few electron microscopic studies have been performed on sperm release from Sertoli cells [3] and the intratesticular excurrent duct system [4, 5]. We carried out an ultrastructural study on the changes in seminiferous elements and intratesticular efferent ductules during gonadotropin-induced spermiation. Particular emphasis was placed on Sertoli cells adjacent to efferent ductules, and their role in sperm transport is discussed.

MATERIALS AND METHODS

Adult males of *Rana porosa porosa* were collected in early November at Kurihashi, Saitama

Prefecture. The frogs were maintained at $18 \pm 1^\circ\text{C}$ with an artificial photoperiod of 12L:12D. Twenty-five frogs were divided into five groups, i.e., one initial control group, two saline-injected control groups and two gonadotropin-injected groups (sacrificed 20 or 45 min after the injection). In the injected control groups, a single injection of 0.7% saline was administered. Gonadotropin treatment consisted of a single injection of hCG (Puberogen, Sankyo Co., Ltd., Tokyo): 2.0 IU/g body weight.

In preparation for histological observation, the right testes and kidneys were fixed in Bouin's solution, embedded in Paraplast (Sherwood Medical, U.S.A.), and cut serially into 5 μm -thick transverse sections; the sections were then stained with Delafield's hematoxylin and eosin. For electron microscopy, the left testes were fixed for 2 hr in 1% glutaraldehyde-1% paraformaldehyde that had been adjusted to pH 7.2 with 0.07 M cacodylate buffer. After being rinsed in the buffer, they were postfixed in cold 1% OsO_4 for 2 hr, dehydrated through a series of ethanols, and embedded in epoxy resin. Thick sections were cut, stained with toluidine blue, and examined with a light microscope to identify sperm transport into efferent ductules. Gold to silver sections were cut with a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead citrate, and examined with an H-300 electron microscope (Hitachi, Ltd.,

Japan).

From the electron microscopical observation, the number of dilated Sertoli cells adjacent to efferent ductules was recorded for each animal, i.e., the seminiferous tubules adjacent to efferent ductules were selected at random, and twenty Sertoli cells in this region were examined. From these results, the percentage of dilated Sertoli cells

was calculated for each group. The results obtained were tested for statistical significance using Student's *t*-test.

RESULTS

Light microscopic observations

In the control frogs, the seminiferous tubules

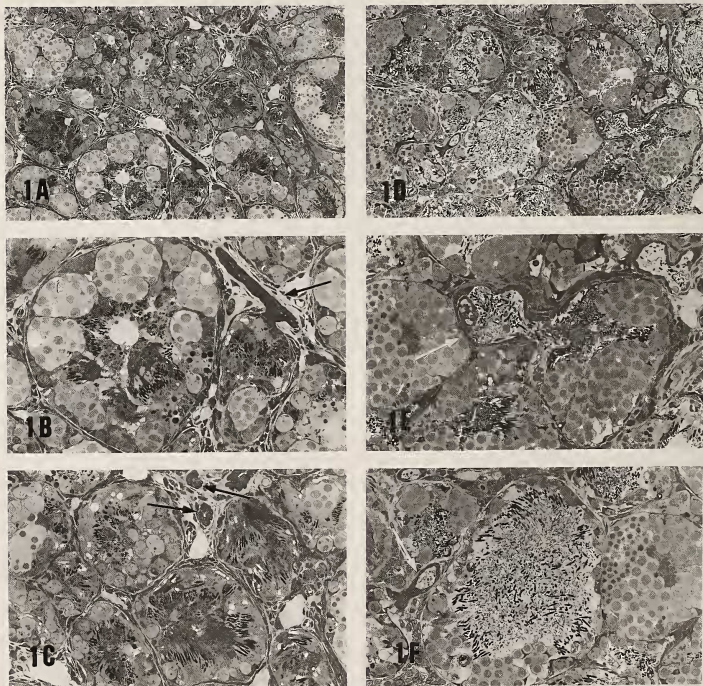
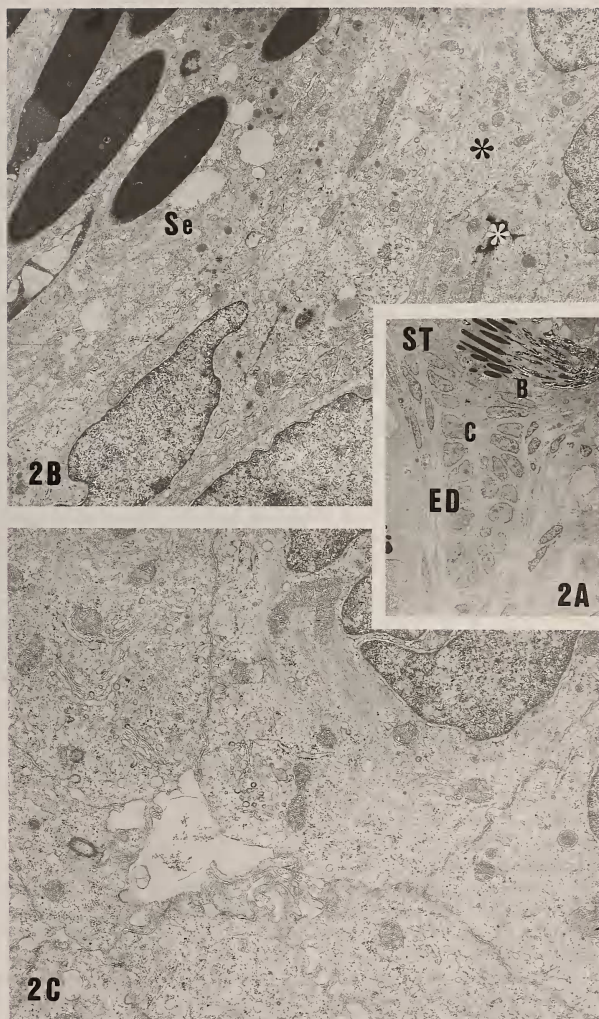


FIG. 1. Testicular histology at the time of spermiogenesis induced by hCG treatment. arrow: intratesticular efferent ductules. A, B, C: control frogs. D, E, F: hCG-treated frogs 20 min after treatment. The release of numerous spermatozoa and their transport from the seminiferous tubules to the efferent ductules can be seen. A and D, $\times 105$; B, C, E and F, $\times 220$.

FIG. 2. A: The region adjoining the seminiferous tubule (ST) and the efferent ductule (ED) in the control. $\times 700$. B: Higher magnification of region B in Fig. 2A. Sertoli cells (Se) are in close contact with the cells of efferent ductules (*). $\times 72000$. C: Higher magnification of the region C in Fig. 2A showing the characteristic abundant filaments in the cells of the efferent ductules. $\times 13200$.



were filled with numerous spermatozoa and cysts of spermatogonia, spermatocytes, and spermatids. Spermatozoa were attached to the Sertoli cells, and free ones were hardly seen in the lumina of the tubule (Fig. 1A, C). Intratesticular efferent ductules were easily distinguished from other testicular components (Fig. 1B, C). The part of the efferent ductules adjacent to the seminiferous tubules was constricted and contained no spermatozoa in their small lumina (Fig. 1B). On the other hand, in the hCG-treated frogs, the lumina of seminiferous tubules were remarkably expanded, and numerous spermatozoa were observed in the lumina 20 min after injection (Fig. 1D, F). Furthermore, spermatozoa were frequently seen in the efferent ductules (Fig. 1E). The expansion of ductule lumina in which no spermatozoa were seen, was also observed. These changes were recognized in 40% of the planes of sections 20 min after hCG treatment and in almost all the planes of sections 45 min after treatment. The appearance of spermatozoa in the Wolffian duct was first recognized 20 min after hCG injection.

Ultrastructural observations

From light and electron microscopical observations, we determined that the intratesticular excurrent duct system is composed only of intratesticular efferent ductules (e.g., Fig. 2).

In the control frogs, Sertoli cells adjacent to efferent ductules were in close contact with the cells of efferent ductules, and no tubule lumen was linked with the small lumina of efferent ductules (Fig. 2B). The cells lining the small lumina of these efferent ductules were interdigitated (Fig. 2C).

When spermatozoa in the hCG-treated frogs were released from the Sertoli cells and observed in the efferent ductules, Sertoli cells adjacent to efferent ductules showed noticeable changes in structure, i.e., elongation of the cytoplasmic processes (Figs. 3A, 4) and dilation of the cell body (Fig. 3B). The percentage of dilated Sertoli cells in this region increased with time after hCG treatment ($p < 0.01$, Table 1). When the expansion of lumina was seen in the cells of efferent ductules, the protrusion of cytoplasmic processes into the lumina was observed (Fig. 5A). Furthermore,

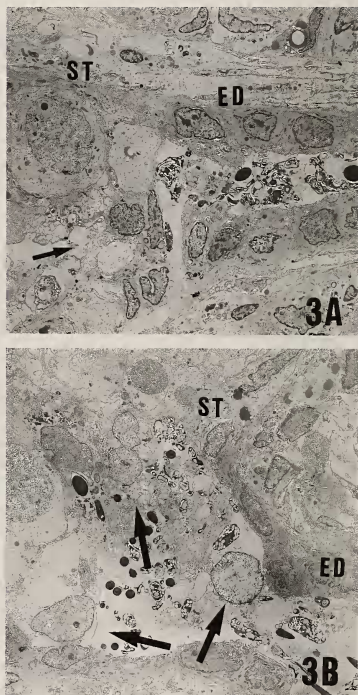


FIG. 3. The region adjoining the seminiferous tubule (ST) and the efferent ductule (ED) after hCG treatment. A: In the Sertoli cells, elongation of a cytoplasmic process (arrow) is observed. B: Arrows indicate dilated Sertoli cells. A, B, $\times 1300$.

these processes were often in contact with residual bodies (Fig. 5B). In the present study, however, phagocytosis of residual bodies by these cells was not seen. These changes are revealed schematically in Figure 6.

In the peritubular parts of the seminiferous tubules and efferent ductules, myoid-like cells and innervation were not observed.

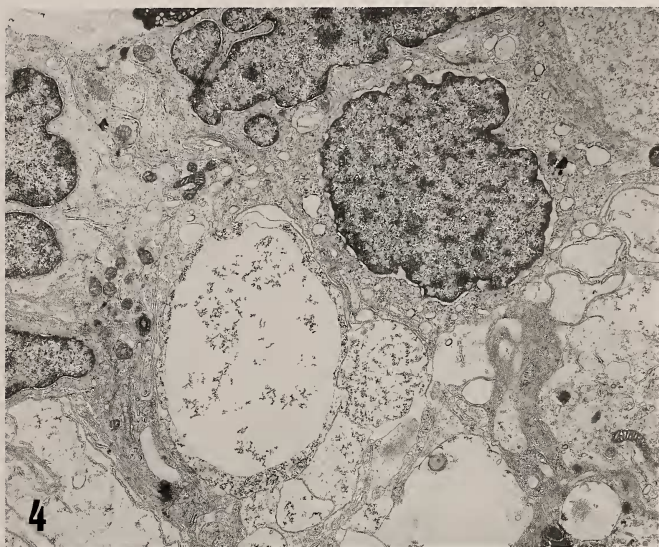


Fig. 4. Sertoli cells after hCG treatment. Sertoli cells show noticeable structural change. $\times 7400$.

TABLE 1. The percentage of dilated Sertoli cells in the region adjoining the seminiferous tubules and the efferent ductules

Group	Time after hCG treatment (min)		
	0	20	45
Control	13.3 ± 3.2	16.7 ± 5.2	6.3 ± 4.2
HCG-treatment	—	$40.4 \pm 7.3^*$	$76.9 \pm 6.8^*$

* $p < 0.01$ as compared with control value.

DISCUSSION

In anurans, numerous studies have been undertaken to determine the mechanism of spermiation [6]. However, there are few reports on the intratesticular excurrent duct system in the process of sperm transport from the seminiferous tubules to the efferent ductules.

In the present study, a remarkable expansion of the lumina of seminiferous tubules and the dilation

of Sertoli cells were seen after hCG-treatment. Shortly after, the release of sperm from the Sertoli cells was observed, and the dilation of the Sertoli cells adjacent to the efferent ductules caused a full opening of the lumina in both tubules and ductules (see Fig. 6).

It is known that a remarkable expansion of the lumina of seminiferous tubules at the time of spermiation indicates fluid accumulation [6]. In the present study, a remarkable expansion of the

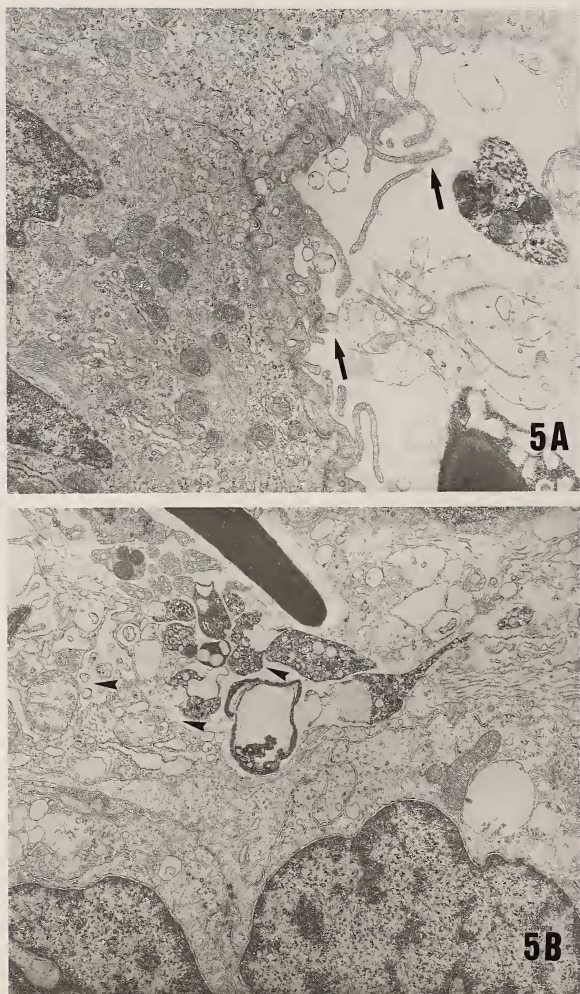


FIG. 5. The cells of the efferent ductules after hCG treatment. A: Arrows indicate the protrusion of cytoplasmic processes into the lumina. B: The cytoplasmic processes are in contact with residual bodies (arrowheads). A, B, $\times 143000$.

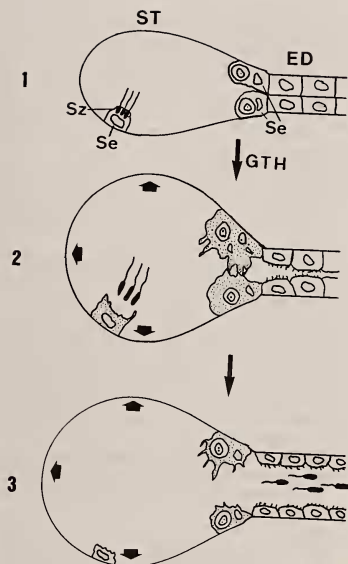


Fig. 6. Schematic representation of changes in the region adjoining the seminiferous tubules and the efferent ductules during sperm transport. This process is divided into three stages. ST: seminiferous tubule, ED: efferent ductule, Se: Sertoli cell, Sz: spermatozoa, GTH: gonadotropin. Stage 1: Seminiferous tubule and efferent ductule in the control are shown. Stage 2: After GTH treatment. Expansion of the seminiferous tubule (large arrows) and dilation of Sertoli cells are seen. Stage 3: Sertoli cells adjacent to the ED dilate, and the lumina of the ST and ED are linked. As a result, sperm transport from the ST to ED takes place.

lumina in seminiferous tubules was observed after hCG treatment. As mentioned above, fluid accumulation in the seminiferous tubules at the time of spermiation occurred simultaneously at the time of the dilation of Sertoli cells. Burgos and Vital-Calpe [3] indicated that fluid accumulation was caused by the dilation of sperm-supporting Sertoli cells at the time of spermiation. In young frogs, fluid accumulation is caused by the application of gonadotropin [7, 8]. In the present study, the

relation between the dilation of Sertoli cells and fluid accumulation in seminiferous tubules is not clear. Further study will be necessary with regard to the mechanism of fluid accumulation.

Burgos and Vital-Calpe [3] reported in *Bufo arenarum* that at the time of hCG-induced spermiation, the dilation of sperm-supporting Sertoli cells could be observed, with the consequence of sperm release from the Sertoli cells. In anurans, however, noticeable cytoplasmic changes are not known to occur in Sertoli cells except in those of the sperm-supporting type. In the present study, the changes in sperm-supporting Sertoli cells after hCG treatment are in accord with Burgos and Vital-Calpe [3]. Furthermore, the dilation of the Sertoli cells adjacent to efferent ductules after hCG treatment indicates that noticeable cytoplasmic changes also occur in Sertoli cells other than the sperm-supporting type. Our findings suggest that these cytoplasmic changes may be caused by gonadotropin treatment irrespective of the stages of the supporting germ cells.

In mammals, the intratesticular excurrent duct system is composed of the rete testis, tubuli recti, and the terminal segment of the seminiferous tubules [1]. The terminal segment of the seminiferous tubules is composed of modified Sertoli cells, and germ cells supported by Sertoli cells are not seen in this region. With regard to the function of the excurrent duct system during spermiation, Wrobel *et al.* [2] reported that in bulls, the terminal segment of the seminiferous tubules functions as a valve-like device to prevent a reflux of spermatozoa and tubuli rectes fluid under normal conditions. Although in anurans, there have been no suggestions as to the function of the excurrent duct system during spermiation, our observations suggest that the germ cell-supporting Sertoli cells adjacent to efferent ductules function as a valve-like device in the transport of spermatozoa which are released into the lumina of seminiferous tubules during spermiation.

With regard to the cells of efferent ductules, Unsicker [4] reported from electron microscopical observations in three anuran species that these cells were very filamentous and involved in mechanical support rather than serving as a contractile aid in sperm transport. Furthermore, innervation

in these peritubular tissues was not recognized [5]. In the present study, the cells of the efferent ductules were filamentous, and innervation in these peritubular tissues was not seen. From these facts, it is difficult to imagine that the cells of the efferent ductules are an active contractile aid to sperm transport in anurans. Therefore, we must conclude that the parts of intratesticular excurrent duct system involved in sperm transport in anurans differ from those in mammals.

In mammals, the epithelial cells of the intratesticular excurrent duct system phagocytose sperm material [2, 9, 10, 11]. From such observations, these investigators have suggested that the epithelial cells were capable of selectively disposing degraded sperm material. Although the processes of efferent ductule cells were frequently in contact with sperm residual bodies at the time of sperm transport in our study, we do not know whether or not efferent ductule cells phagocytose sperm residual bodies.

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